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There are four major findings from the three years of work devoted to the effects of chronic JP-8 jet fuel exposure on the lungs and secondary organs. These fundings are the following - (1) Chronic exposure to JP-8 jet fuel alters pulmonary function and lung structures with an acute response with as little as seven days of low dose, approximately 500 mg/m3, exposure to JP-8 jet fuel. (2) Chronic exposure to JP-8 jet fuel increased liver, spleen, and kidney weights compared to controls. Microscopic evaluation of liver sections were normal; however, kidney and spleen had histological changes consistent with organic solvent exposure. (3) There is a correlation between JP-8 jet fuel exposure-induced decreases in lung Substance P levels and lung neutral endopeptidase levels. Chronic exposure to JP-8 jet fuel caused a decrease in lung Substance P levels with a corresponding increase in lung neutral endopeptidase levels. (4) There is a recovery process in the 56 day low dose JP-8 jet fuel-exposed lungs as marked by a return to baseline and longitudinal control 99mTcDTPA values. The 99mTcDTPA data was very consistent with our pathologic findings of very little lung injury in the 56 day low dose JP-8 jet fuel-exposed rats. We speculate that this finding indicates that there is a "threshold" level of JP-8 jet fuel exposure that the lungs defense mechanism(s) can tolerate.

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THE CHRONIC EFFECTS OF JP-8 JET FUEL EXPOSURE ON THE LUNGS

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Prepared For- Dr. Kozumbo

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SUMMARY ABSTRACT

There are four major findings from the three years of work devoted to the effects of chronic JP-8 jet fuel exposure on the lungs and secondary organs. These findings are the following-

(1) Chronic exposure to JP-8 jet fuel alters pulmonary function and lung structures with an acute response with as little as seven days of low dose, approximately 500 mg/m^3, exposure to JP-8 jet fuel.

(2) Chronic exposure to JP-8 jet fuel increased liver, spleen, and kidney weights compared to controls. Microscopic evaluation of liver sections were normal; however, kidney and spleen had histological changes consistent with organic solvent exposure.

(3) There is a correlation between JP-8 jet fuel exposure-induced decreases in lung Substance P levels and lung neutral endopeptidase levels. Chronic exposure to JP-8 jet fuel caused a decrease in lung Substance P levels with a corresponding increase in lung neutral endopeptidase levels.

(4) There is a recovery process in the 56 day low dose JP-8 jet fuel-exposed lungs as marked by a return to baseline and longitudinal control 99mTcDTPA values. The 99mTcDTPA data was very consistent with our pathologic findings of very little lung injury in the 56 day low dose JP-8 jet fuel-exposed rats. We speculate that this finding indicates that there is a "threshold" level of JP-8 jet fuel exposure that the lungs' defense mechanism(s) can tolerate.

STATEMENT OF WORK

There will be a total of 372 rats utilized in the study. The rats will be divided into the following groups-

(1) Baseline Control, (N=12). These rats will be killed at the start of the study to establish baseline values on all the parameters to be examined in the study.
(2) Longitudinal Control, (N=180). These rats will undergo exposure to sham air.
(3) JP-8 Jet Fuel-Exposed, (N=180). These rats will be exposed to either of the three concentrations of JP-8 jet fuel (30 mg/m^3, 300 mg/m^3, or 1020 mg/m^3) for one of the four exposure time periods (1 day, 7 days, 28 days, or 56 days).

The parameters we will study are the following-

(1) ^{99m}TcDTPA pulmonary epithelial permeability of each rat.
(2) Lung mechanics of dynamic and static lung compliance, work of breathing, power of breathing, respiratory time constant, and lung resistance on each rat.
(3) Measure lung eicosanoids, TNF, IL-1, and Substance P in nine rats in each group.
(4) Pathologic studies of wet lung weight/body weight ratio, electron and light microscopy in three rats in each group.
(5) Alveolar macrophage studies in nine rats in each group.

(6) Pharmacological blocker studies of Substance P, N=6 for each JP-8 jet fuel exposure group and their corresponding longitudinal control group, N=6.

The proposed study will take three years to complete. We believe it is essential to standardize our study timetable as much as possible to minimize the effects of variables such as seasonal variations of temperature, humidity, and pollen count on our rat population. Consequently, we propose to complete 124 rats/year for the three years of the study with equal numbers of rats from each group completed in each of the yearly time sequences.

PUBLICATIONS FROM PROJECT: June 2, 1994

(1) Witten ML, Pfaff JK, Lantz RC, Parton KH, Chen H, Hays A, Kage R, Leeman SE: Capsaicin pretreatment before JP-8 jet fuel exposure causes a large increase in airway sensitivity in rats. REGULATORY PEPTIDES, 1992, S1:S176.

(2) Witten ML: Chronic effects of JP-8 jet fuel exposure on the lungs. GOVERNMENT REPORTS, ANNOUNCEMENTS, & INDEX, Issue 17, 1992.

(3) Witten ML, Pfaff JK, Parton K, Lantz RC, Carter D, Leeman SE: JP-8 jet fuel exposure alters lung chemical mediator and substance P activity in rats. THE FASEB JOURNAL, 1992, 6:A1065.

(4) Chen H, Witten ML, Pfaff JK, Lantz RC, Carter D: JP-8 jet fuel exposure increases alveolar permeability in rats. THE FASEB JOURNAL, 1992, 6:A1064.

(5) Pfaff J, Erickson R, Lantz R, Witten M: Influence of aryl hydrocarbon hydroxylase activity on lung injury from JP-8 jet fuel exposure in the congenic mouse. THE FASEB JOURNAL, 1992, 6:A1065.

(6) Witten ML, Grad R, Quan SF, Lantz RC, Sobonya RE, Lemen RJ: Effects of respiratory viruses on pulmonary alveolar macrophages. PEDIATRIC PULMONOLOGY, 1992, 12:105-112.

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(8) Parton KH, Pfaff J, Hays AM, Witten M: Effects of JP-8 jet fuel inhalation on the liver of F-344 rats. THE TOXICOLOGIST, 1993, 13:83.

(9) Pfaff J, Parliman G, Parton K, Lantz R, Chen H, Hays A, Witten M: Pathologic changes after JP-8 jet fuel inhalation in Fischer 344 rats. THE FASEB JOURNAL, 1993, 7:A408.

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(11) Hays AM, Tollinger BJ, Tinajero JP, Robledo RF, Lantz RC, Witten ML: Changes in lung permeability after chronic exposure to JP-8 jet fuel. THE FASEB JOURNAL, 1994, 8:A122.

(12) Parton KH: The effects of JP-8 jet fuel inhalation on liver and kidney function in male F-344 rats. Master's Thesis in the Department of Pharmacology/Toxicology, University of Arizona, that was approved on May 5, 1994.

(13) Pfaff JK, Parton K, Lantz RC, Chen H, Hays AM, Witten ML: Inhalation exposure to JP-8 jet fuel alters pulmonary function and Substance P levels in Fischer 344 rats. JOURNAL OF APPLIED TOXICOLOGY (in press).

(14) Pfaff JK, Parton K, Lantz RC, Chen H, Hays AM, Leeman SE, Witten ML: Neutral endopeptidase (NEP) and its role in pathologic pulmonary change with inhalation exposure to JP-8 jet fuel. REGULATORY PEPTIDES (submitted).

(15) Hays AM, Parliman G, Tollinger BJ, Tinajero JP, Robledo RF, Lantz RC, Witten ML: Changes in lung permeability correlate with pathology after chronic exposure to JP-8 jet fuel. EXPERIMENTAL LUNG RESEARCH (in preparation).

PARTICIPATING PROFESSIONALS

(1)	Mark L. Witten, Ph.D. University of Arizona College of Medicine	Principal Investigator
(2)	Susan E. Leeman, Ph.D. Boston University College of Medicine	Consultant
(3)	Robert C. Lantz, Ph.D. University of Arizona College of Medicine	Co-Investigator
(4)	Dean E. Carter, Ph.D. University of Arizona College of Pharmacy	Consultant
(5)	John K. Pfaff, M.D. Lt. Commander, U.S. Navy Medical Corps	Fellow
(6)	Kathy H. Parton, D.V.M. University of Arizona College of Pharmacy	Master's Student
(7)	Huizhong Chen, M.D. Jiangxi Medical College, Nanchang, China	Visiting Scientist
(8)	Richard J. Lemen, M.D. University of Arizona College of Medicine	Consultant

(9) Dr. James R. Halpert — Consultant
University of Arizona College of Pharmacy

(10) Richard E. Sobonya, M.D. — Consultant
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(11) Brian J. Tollinger — Research Associate
University of Arizona College of Pharmacy

(12) Allison M. Hays — Research Associate
University of Arizona College of Medicine

(13) Robert P. Erickson, M.D. — Consultant
University of Arizona College of Medicine

Advanced Degrees Awarded:

(1) Dr. Parton was awarded a Master's degree in the Department of Pharmacology and Toxicology, College of Pharmacy, University of Arizona, on May 5, 1994.

COUPLING ACTIVITIES

We have assisted Captain Donald R. Tocco, a research toxicologist, from the Armstrong Aerospace Medical Research Laboratory at Wright-Patterson Air Force Base in setting up his laboratory to perform ^{99m}TcDTPA pulmonary clearance studies and computerized pulmonary function tests in rats. Dr. Tocco and his associate visited our laboratory in March of 1994 to learn how to set up his PEDS-LAB computerized pulmonary function system.

DISCOVERIES, INVENTIONS, PATENT DISCLOSURES, AND SPECIFIC APPLICATIONS

There have been no discoveries, inventions, patent disclosures and specific applications generated from the Air Force project at this point in time.

RESEARCH ACCOMPLISHMENTS

The following data is presented in group form with the groups corresponding to-

Group 1	Low Dose 7 Day JP-8 Jet Fuel Exposure (N=23)
Group 2	Baseline Controls (N =21)
Group 3	Low Dose 28 Day JP-8 Jet Fuel Exposure (N=15)
Group 4	Longitudinal 7 Day Controls (N=9)
Group 5	Longitudinal 28 Day Controls (N=17)
Group 6	High Dose 7 Day JP-8 Jet Fuel Exposure (N=14)
Group 7	High Dose 28 Day JP-8 Jet Fuel Exposure (N=20)
Group 8	Low Dose 56 Day JP-8 Jet Fuel Exposure (N=11)

Group 9 High Dose 56 Day JP-8 Jet Fuel Exposure (N=11)
Group 10 Longitudinal 56 Day Controls (N=11)

Pulmonary Epithelial Permeability as measured by 99mTcDTPA Lung Clearance

Group	99mTcDTPA k value
1	2.16 (0.24)
2	1.61 (0.19)
3	2.51 (0.35)
4	1.52 (0.20)
5	1.20 (0.17)
6	2.07 (0.18)
7	1.95 (0.36)
8	1.04 (0.13)
9	2.52 (0.48)
10	0.99 (0.15)

$p < 0.05$ between-

Group 1 -vs- Groups 5, 8, 10
Group 2 -vs- Groups 3,9
Group 3 -vs- Groups 4,5,8,10
Group 4 -vs- Group 9
Group 5 -vs- Groups 6,7,9
Group 6 -vs- Groups 8,10
Group 7 -vs- Groups 8,10
Group 8 -vs- Group 9
Group 9 -vs- Group 10

Pulmonary Resistance ($cmH_2O/L/sec$)

Group	Pulmonary Resistance
1	402 (25)
2	367 (36)
3	372 (39)
4	314 (16)
5	365 (57)
6	147 (22)
7	293 (9)
8	245 (8)
9	182 (7)
10	183 (11)

$p < 0.05$ between-

Group 1 -vs- Groups 4,6,7,8,9,10
Group 2 -vs- Groups 6,7,8,9,10

Group 3 -vs- Groups 6,7,8,9,10
Group 4 -vs- Groups 6, 9,10
Group 5 -vs- Groups 6,9,10
Group 6 -vs- Group 7
Group 7 -vs- Groups 9,10

Dynamic Lung Compliance (ml/cmH_2O)

Group	Dynamic Lung Compliance
1	0.15 (0.02)
2	0.13 (0.01)
3	0.12 (0.01)
4	0.12 (0.01)
5	0.14 (0.01)
6	0.22 (0.02)
7	0.14 (0.02)
8	0.04 (0.002)
9	0.25 (0.01)
10	0.34 (0.02)

$p < 0.05$ between-

Group 1 -vs- Groups 6,8,9,10
Group 2 -vs- Groups 6,8,9,10
Group 3 -vs- Groups 6,8,9,10
Group 4 -vs- Groups 6,8,9,10
Group 5 -vs- Groups 8,9,10
Group 6 -vs- Groups 7,8,10
Group 7 -vs- Groups 8,9,10
Group 8 -vs- Groups 9,10
Group 9 -vs- Group 10

Wet Lung Weight (grams)

Group	Wet Lung Weight
1	1.23 (0.04)
2	1.38 (0.08)
3	1.22 (0.03)
4	1.14 (0.03)
5	1.20 (0.03)
6	1.35 (0.08)
7	1.40 (0.05)
8	1.41 (0.04)
9	1.48 (0.05)
10	1.46 (0.14)

$p < 0.05$ between-

Group 1 -vs- Groups 2,7,8,9,10
Group 2 -vs- Groups 3,4,5
Group 3 -vs- Groups 7,8,9,10
Group 4 -vs- Groups 6,7,8,9,10
Group 5 -vs- Groups 7,8,9,10

Wet Lung Weight/Body Weight Ratio

Group	Wet Lung Wt./Body Wt. Ratio
1	0.006 (0.0002)
2	0.006 (0.0002)
3	0.005 (0.0001)
4	0.005 (0.0001)
5	0.005 (0.0001)
6	0.006 (0.0004)
7	0.006 (0.0002)
8	0.005 (0.0002)
9	0.006 (0.0004)
10	0.006 (0.001)

$p < 0.05$ between-

Group 1 -vs- Groups 3,4,5,7,8,9
Group 2 -vs- Groups 3,8
Group 3 -vs- Group 6
Group 4 -vs- Group 6
Group 5 -vs- Group 6
Group 6 -vs- Group 8
Group 7 -vs- Group 8
Group 8 -vs- Group 10

Lung Substance P Levels (femtomoles/ml BALF)

Group	Substance P Levels
1	9.2 (1.4)
2	4.9 (1.2)
3	13.6 (1.8)
4	40.4 (8.5)
5	60.0 (9.3)
6	0 (0)*
7	0 (0)*
8	0 (0)*
9	0 (0)*
10	23.6 (3.8)

* All values below the 5 femtomoles/ml BALF detectable limit of the SP assay.

$p < 0.05$ between-

Group 1 -vs- Groups 4,5,6,7,10
Group 2 -vs- Groups 4,5,10
Group 3 -vs- Groups 5,6,7,8,9
Group 4 -vs- Groups 5,6,7,8,9,10
Group 5 -vs- Groups 6,7,8,9,10
Group 6 -vs- Group 10
Group 7 -vs- Group 10
Group 8 -vs- Group 10
Group 9 -vs- Group 10

Lung Neutral Endopeptidase (NEP) Levels (millimoles/ml BALF)

Group	NEP
1	0.84 (0.08)
3	0.77 (0.20)
4	1.58 (0.77)
5	0.85 (0.15)
6	1.98 (0.84)
7	3.22 (0.73)
8	0.92 (0.12)
9	1.24 (0.32)
10	0.21 (0.07)

$p < 0.05$ between-

Group 1 -vs- Group 7
Group 2 -vs- Group 7
Group 3 -vs- Group 7
Group 4 -vs- Group 7
Group 5 -vs- Group 10
Group 6 -vs- Groups 8,9,10

Lung Pathology

7 Day Low Dose JP-8 Jet Fuel Exposure

The majority of the lung parenchyma appeared normal. However, the epithelium of the terminal bronchioles did appear thickened. There were cells in the terminal bronchial lumen which resembled either sloughed epithelial cells or macrophages. There were also unrelated, localized areas of parenchyma that demonstrated a slight influx of macrophages.

7 Day High Dose JP-8 Jet Fuel Exposure

There was widespread, acute hemorrhage found in 50% of the lungs processed for histological analysis. The alveolar spaces in these lungs were filled with erythrocytes and fluid. The nearby bronchiole lumen were partially filled with red blood cells and fluid. There appeared to be thickening of the epithelial lining of the terminal bronchioles. There was congestion of the small blood vessels and capillaries. Electron microscopy revealed breaks in the alveolar-capillary membrane and areas of epithelial cell loss. Type II epithelial cells were also found to be vacuolated and in various stages of degeneration.

28 Day Low Dose JP-8 Jet Fuel Exposure

The majority of the lung parenchyma appeared normal. There was apparent epithelial thickening of the terminal bronchioles. Red blood cells were found scattered throughout the alveolar spaces. There were localized areas of parenchyma that demonstrated thickened alveolar septums. Electron microscopy revealed similar degeneration and vacuolization of Type II epithelial cells as those found in the 7 Day High Dose JP-8 jet fuel exposure group. The perivascular sites of small blood vessels demonstrated an influx of leukocytes and evidence of edema. There was no congestion of the capillaries or small blood vessels.

28 Day High Dose JP-8 Jet Fuel Exposure

Most of the lung parenchyma appeared normal. However, scattered localized areas of inflammation were noted in association with small blood vessels, thickened alveolar septums, and terminal and respiratory bronchioles. The epithelium of small bronchioles also appeared to be thickened.

56 Day Low Dose JP-8 Jet Fuel Exposure

The lung parenchyma appeared normal. The perivascular tissue of small blood vessels demonstrated a slight influx of leukocytes with edema. Minute areas of inflammation were scattered in the parenchyma. Thickened epithelium of the bronchioles was still apparent.

56 Day High Dose JP-8 Jet Fuel Exposure

The lung parenchyma appears mostly normal. The perivascular tissue of small blood vessels demonstrated an influx of leukocytes and edema. There were minute areas of inflammation scattered in the parenchyma. The epithelium of the bronchioles was still evident. Electron microscopy showed vacuolization of the Type II epithelial cells.